

In the Claims:

Please amend claims 1, 4, and 33 as set forth below. Please cancel claims 2, 3, 32, and 36 without disclaimer or prejudice to the inclusion of the subject matter contained therein in any later filed continuation or divisional application(s) as set forth below.

1. (Currently amended) A method for producing therapeutic human T regulatory cells (Treg cells) with enhanced suppressor activity, said method comprising:

- selecting a sample of human CD4⁺ T cells;
- contacting said sample with an anti-CD25 antibody;
- isolating cells that bind to said anti-CD25 antibody from said sample using a double column magnetic antibody cell sorting (MACS) purification procedure, to produce an isolated population of human CD4⁺CD25⁺ Treg cells; and
- culture-expanding said population of human CD4⁺CD25⁺ Treg cells comprising contacting said isolated population of human CD4⁺CD25⁺ Treg cells with immobilized anti-CD3 antibody and immobilized anti-CD28 antibody ~~at a predetermined ratio, wherein the ratio of the amount of anti-CD3 antibody to anti-CD28 antibody is less than 1, further culture-expanding said isolated population of human CD4⁺CD25⁺ Treg cells in the presence of a CD4⁺ feeder cell or CD4⁺ feeder cell conditioned medium~~, thereby producing therapeutic human Treg cells with enhanced suppressor activity, ~~wherein said culture expanded Treg cells are CD62L⁺/CD27⁺ and are capable of inhibiting proliferation of CD4⁺CD25⁻ responding T cells in a Mixed Lymphocyte Reaction (MLR) assay by at least 90%.~~

2-3. (Canceled)

4. (Currently amended) The method of claim [[3]] 1, wherein said isolating step comprises contacting the selected human CD4⁺ T cells with 2μl of said anti-CD25 magnetic microbeads per 10⁷ total cells, and wherein the double column purification procedure comprises purifying by running the bead/cell composition over a magnetic column to separate bead-bound cells, washing, and re-eluting over a second magnetic column, and again washing until <1-2% of nonsuppressor cells remain in the purified isolate isolated population of human CD4⁺CD25⁺ Treg cells.

5. (Previously presented) The method of claim 1, wherein said culture-expanding step produces an effective amount of suppressor cells to achieve therapeutic suppression of an immune or autoimmune response in a human.

6. (Canceled)

7. (Previously presented) The method of claim 1, wherein said culture-expanding step further comprises contacting said isolated population of human $CD4^+CD25^+$ Treg cells with IL-2.

8. (Previously presented) The method of claim 1, wherein said isolated population of human $CD4^+CD25^+$ Treg cells are expanded at least 10-20 fold in 14 days of culture in said culture-expanding step.

9. (Previously presented) The method of claim 8, wherein said isolated population of human $CD4^+CD25^+$ Treg cells are expanded at least 100-fold by culturing for an additional 1-2 weeks.

10. (Previously presented) The method of claim 1, further comprising generating therapeutic human Treg cell lines that retain long term down-regulatory suppressor function.

11. (Previously presented) The method of claim 1, wherein the sample of human $CD4^+$ T cells is selected from the group consisting of whole or partially purified blood or hematopoietic cells, selected from the group consisting of peripheral blood mononuclear cells, peripheral blood lymphocytes, spleen cells, tumor-infiltrating lymphocytes and lymph node cells, and bone marrow and peripheral bone marrow cells.

12-27. (Canceled)

28. (Previously presented) The method of claim 1, wherein the ratio of the amount of anti-CD3 antibody to anti-CD28 antibody is at least 1:5.

29. (Previously presented) The method of claim 10, wherein said therapeutic human Treg cell lines retain long term down-regulatory suppressor function for at least three weeks.

30. (Previously presented) The method of claim 1, wherein said anti-CD25 antibody is directly conjugated to a magnetic microbead.

31. (Previously presented) The method of claim 1, wherein said MACS purification procedure is an indirect method, wherein said isolating step further comprises contacting said sample to magnetic microbeads conjugated to a secondary agent that binds to said anti-CD25 antibody.

32. (Canceled).

33. (Currently amended) The method of claim [[32]] 31, wherein said isolating step further comprises substantially enhancing CD4⁺CD25^{bright} cells in said isolated population, while substantially depleting CD25^{dim} cells in said isolated population.

34. (Previously presented) The method of claim 33, wherein said isolating step comprises contacting the selected, anti-CD25 antibody-contacted human CD4⁺ T cells with 2 μ l of said magnetic microbeads per 10⁷ total cells, and wherein the double column purification procedure comprises purifying by running the bead/cell composition over a magnetic column to separate bead-bound cells, washing, and re-eluting over a second magnetic column, and again washing until <1-2% of nonsuppressor cells remain in the purified isolate.

35. (Previously presented) The method of claim 31, wherein said anti-CD25 antibody is conjugated to FITC and said secondary agent is an anti-FITC antibody.

36. (Canceled).